RETROVIRUSES

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DEDICATION

HOMAGE TO HOWARD TEMIN

It is deeply sad that one voice is missing from this book, that of Howard Temin. He died in early 1994 at age 59. He was a pioneer. I want to take a few pages to point out that the modern history of retrovirology depended on two critical contributions by Howard. These discoveries are described in more detail in the first chapter by Peter Vogt.

Along with Harry Rubin, Howard devised the first cell culture focus-forming assay for a retrovirus, the Rous sarcoma virus. This work opened up the study of retroviruses in cell culture, providing the first truly quantitative assay of viral transformation and the first quantitative assay for a retrovirus (although in 1958, the concept of a retrovirus was still to be developed).

Howard's second major contribution was to realize that the ability of Rous sarcoma virus to stably transform cells implied that this RNA virus must transfer its genetic information to DNA—otherwise, he reasoned, how could transformation be so stable? This latter insight was first presented in his Caltech thesis in 1960 and became his personal research challenge over the next 10 years. In 1964, it was enshrined as the provirus hypothesis, an explicit proposal that an integrated copy of viral DNA maintained the genetic stability of a retroviral infection. Only when Howard and I were able to demonstrate in 1970 that the virions of retroviruses have a reverse transcriptase was the notion widely accepted in the scientific community.

Ten years in the scientific wilderness is a long time; few have had to bear the silence of their colleagues for so long. I can remember meetings in the 1960s when Howard would present his latest data supporting the provirus notion only to be greeted by either skeptical questions or quiet, polite disbelief. Howard's conviction that there had to be a provirus never seemed to waver over the whole decade. He knew he was right—and he was—but what fortitude it took

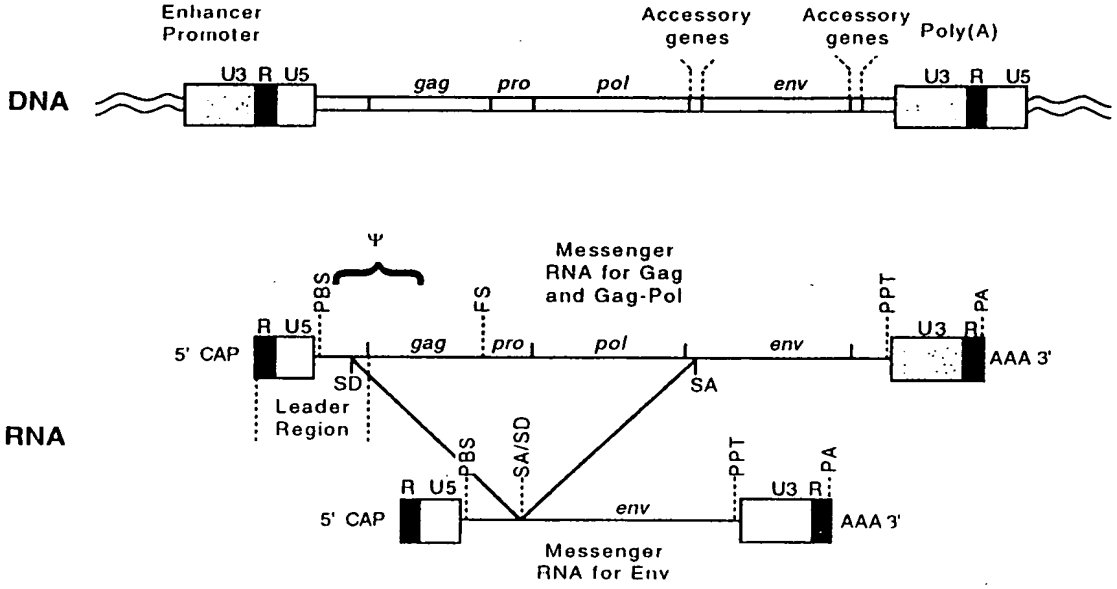

Figure 4 Genetic organization of generalized provirus. The proviral DNA as it is inserted into host DNA is shown at the top, with the long terminal repeats (LTRs) composed of U3, R, and U5 elements at each end abutting cellular sequences. Sequences in the LTR that are important for transcription, for example, enhancers, the promoter, and the poly(A) addition signal, are marked. The *gag*, *pro*, *pol*, and *env* sequences are located invariably in the positions shown in all retroviruses. Accessory genes are located as shown, and also overlapping *env* and U3 and each other, and occasionally in other locations. The RNA that is the primary transcriptional product is shown on the second line. Sequences that are important for replication and gene expression are shown in the approximate locations in which they are typically found. (PBS) Primer-binding site; (ψ) encapsidation sequence; (SD) splice donor site; (FS) frameshift site; (SA) splice acceptor site; (PPT) polypurine tract; (PA) polyadenylation signal; (AAA) poly(A) tail. The spliced messenger RNA for the Env protein is shown on the third line. Retroviruses with accessory genes have other spliced mRNAs and thus other splice donor and splice acceptor sites as well.

ter 3). A few retroviral groups also contain another gene, called *dut*, which encodes a deoxyuridine triphosphatase (dUTPase or DU). Unlike all other genes, *dut* is found in differing locations, being translated in the *pro* reading frame in the B- and D-type viruses, and in the *pol* frame in the nonprimate lentiviruses.

In addition to *gag*, *pro*, *pol*, and *dut*, those retroviruses classified as complex—the lentivirus genus, the spumavirus genus, the HTLV/bovine leukemia virus (BLV) genus, and a newly characterized fish virus genus—also contain "accessory" genes. Accessory genes regulate and coordinate viral gene expression, and some also have other ancillary roles. These genes are located between *pol* and *env*, just downstream from *env* including the U3 region of the LTR, or overlapping portions of *env* and each other.

Some retroviruses carry genes of a different class: the oncogenes, or *onc* genes. Many retroviruses—hereafter referred to as "transforming" viruses—were first identified by their ability to rapidly cause tumors in animals and oncogenically transform cells in culture. Transformation of cultured cells invariably was traced to host-derived sequences that the virus had acquired (Chapter 10). With the exception of some strains of RSV, retroviruses that carry oncogenes are defective, having suffered variable deletions of one or more of the viral genes needed for replication during or after the acquisition event. As a consequence, many retroviral oncogenes are expressed as Gag-Onc fusion proteins, with part or most of *gag* being deleted. There are also numerous examples in which the oncogene replaces *env* or is positioned elsewhere in the genome. Retroviruses with such rearrangements are defective for replication on their own and can replicate only if the cell is also infected with a nondefective virus, usually called a helper virus. In the nondefective strains of

Table 1 Retroviral Genes

Gene	Properties/function of protein
Common to All Retroviruses	
gag	precursor to internal structural proteins
pro	PR enzyme
pol	precursor to RT and IN enzymes
env	precursor to envelope glycoproteins
Accessory Genes	
HTLV/BLV (e.g., HTLV-1)	
tax	transcription activator
rex	splicing/RNA transport regulator
Lentiviruses (for primate lenti, e.g., HIV-1)	
tat	activates transcription
rev	regulates splicing/RNA transport
vif	affects infectivity of viral particles
vpr and/or vpx	is present in virion; has nuclear localization signal; facilitates infectivity in quiescent cells; triggers CD4 endocytosis, alters signal transduction in T cells; enhances virion infectivity
nef	
vpu	integral membrane protein; triggers CD4 degradation; enhances virion release
dut	dUTPase (only in nonprimate lentiviruses); facilitates replication in certain cell types
Type B (e.g., MMTV)	
sag	superantigen
dut	dUTPase (NC-DU fusion)
Type D (e.g., M-PMV)	
dut	DU enzyme (NC-DU fusion)
Spumaviruses (e.g., HSRV)	
bel1	activates transcription
bel2	?
bet	?
Piscine retroviruses (e.g., WDSV)	
orf A	?
orf B	?
orf C	?

RSV, the v-*src* oncogene is freestanding downstream from *env* and is expressed from a separately spliced mRNA (Chapter 6). An overview of the organization of genes in a few prototypic retroviruses is presented in Figure 5.

The genes in the viral DNA are bracketed by the long terminal repeats (LTRs), identical sequences that can be divided into three elements. U3 is derived from the sequence unique to the 3′end of the RNA, R is derived from a sequence repeated at both ends of the RNA, and U5 is derived from the sequence unique to the 5′end of the RNA. The genesis of the LTR elements lies in the process of reverse transcription, whereby the enzyme "jumps" from one end of the template to the other (Chapter 4). The sizes of these three elements vary considerably among different retroviruses, with U3 typically ranging from several hundred nucleotides to more than a thousand nucleotides, R from a dozen to more than a hundred nucleotides, and U5 from about one to two hundred nucleotides (Table 2). From the definition of U3, R, and U5, it follows that the site of transcription initiation is at the boundary between U3 and R, and the site of poly(A) addition is at the boundary between R and U5, as shown. The other boundaries of U3 and U5 are determined by the sites of initiation of plus- and minus-strand DNA synthesis. U3 contains most of the transcriptional control elements of the provirus, which in-